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14. ABSTRACT The contribution of NF-kappaB signaling within macrophages to metastatic potential has not been directly investigated in vivo. We use novel transgenic models to address this question during breast to lung metastasis. Expression of an inhibitor (DN) or an activator (IKK) of NF-kappaB is induced by crossing either inducible transgenic with a second transgenic expressing the reverse transactivator (rtTA) protein in macrophages and administering doxycycline (dox) in drinking water. We have modulated NF-kappaB activity in macrophages and tested effects on breast cancer metastasis to the lung. Using a tail vein model

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NF-kappaB, macrophages, doxycycline-inducible transgenes, metastasis

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INTRODUCTION

Both macrophages and NF-kappaB (NF-κB) signaling are known to be important in mammary tumorigenesis. However, the contribution of NF-κB signaling within the macrophages to metastatic potential has not been directly investigated *in vivo*. We proposed to use transgenic models in a novel combination to address this critically important question related to breast to lung metastasis. Expression of an inhibitor (DN) or an activator (IKK) of NF-κB can be induced by crossing either with a transgenic expressing the reverse transactivator (rtTA) protein in a specific cell type and administering doxycycline (dox) in drinking water (1). The rtTA protein has been targeted to macrophages (2). A third type of transgenics (NGL), act as an *in vivo* reporter of NF-κB activity and indicates overall inflammatory response (3). We have these transgenics and have combined them to modulate NF-κB activity in macrophages and to test the impact of this altered microenvironment on breast cancer metastasis to the lung. Our hypothesis was that NF-κB activity in macrophages determines metastatic potential and thus represents a target for inhibition of metastatic breast cancer. Many of the tumor-promoting mediators expressed by macrophages are under the transcriptional control of NF-κB. Elevated NF-κB activity is an integrator of inflammatory responses and is strongly correlated with tumors. An inflammatory microenvironment promotes increased metastasis. Therefore, it is likely that NF-κB activity within macrophages is a critical component of mammary metastasis, but this question had not been addressed directly. We designed a strategy to modulate NF-κB in macrophages *in vivo* and determine the impact of these changes on mammary to lung metastasis. When we commenced these studies we were expecting that increasing NF-κB in macrophages would skew them towards a pro-tumor tumor-associated macrophage (TAM) phenotype. We were predicting that these macrophages would induce a chronic inflammatory environment that would support tumor metastasis by increasing angiogenic and matrix remodeling signals. We proposed to address two questions. 1) Does activation of NF-κB in macrophages contribute to mammary metastasis? 2) Can inhibition of NF-κB in macrophages inhibit mammary to lung metastasis?

BODY

Task 1. Determine effects of increased NF-κB activity in macrophages on mammary to lung metastasis:

Determine effects of increased NF-κB activity in macrophages on lung tumor development using a tail vein metastasis model.

These studies were designed to introduce breast tumor cells via the tail vein such that they metastasize to the lungs in mice in which we can inducibly activate NF-κB in macrophages. This is achieved by crossing homozygous c-fms-rtTA transgenics with heterozygous IKK transgenics to generate double IKFM transgenics plus c-fms-rtTA control littermates. When IKFM mice are treated with doxycycline (dox) provided at 2mg/ml in 5% sucrose drinking water *ad lib*, expression of the constitutive form of the IKK2 activator of NF-κB is targeted to macrophages. Experimental subsets include IKFM and control littermates for this and all following dox induction experiments. Polyoma-derived breast cancer cell lines were introduced via the tail vein into immunocompetent IKFM and control mice. Dox treatment started 1 week prior to cell injection and continued until harvest at 2 weeks (PY1) post cell injection. At the end point lungs were harvested and the total number of visible lung metastases on the surface were counted. Our original hypothesis suggested that activation of NF-κB would have pro-tumor effects resulting in greater numbers of lung metastases. We were therefore surprised when our data showed the opposite effect with fewer metastases in IKFM mice as compared with control littermates (Figure 1). As this result was unexpected we repeated the study and obtained the same highly significant observation (data not shown).

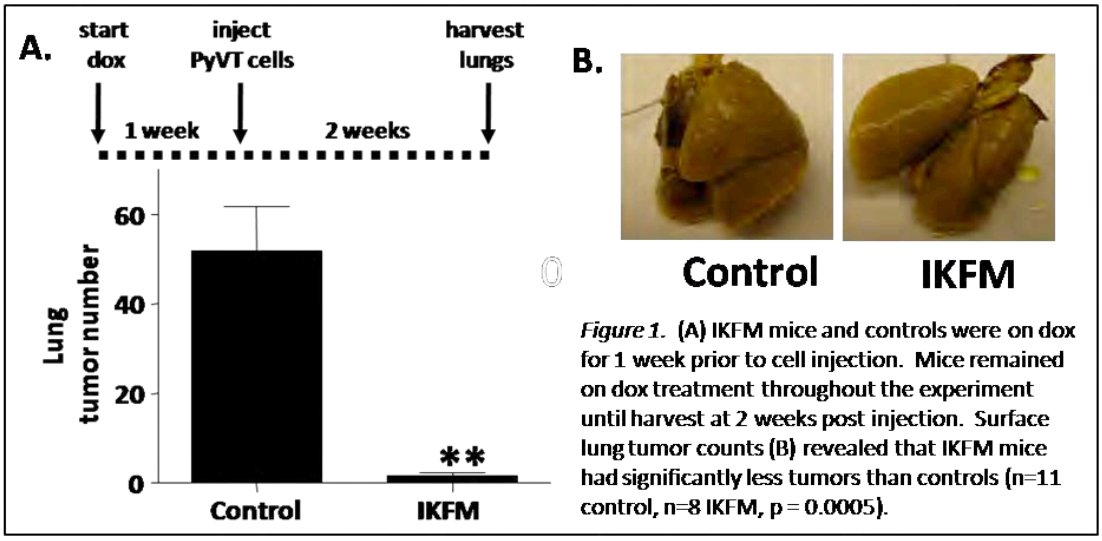


Figure 1. (A) IKFM mice and controls were on dox for 1 week prior to cell injection. Mice remained on dox treatment throughout the experiment until harvest at 2 weeks post injection. Surface lung tumor counts (B) revealed that IKFM mice had significantly less tumors than controls (n=11 control, n=8 IKFM, p = 0.0005).

*Determine effects of increased NF-κB activity in macrophages at **early stages** of metastasis on lung tumors using a tail vein metastasis model.*

Given the unexpected outcome of the studies above in which activation of NF-κB resulted in increased lung metastases we were interested to determine the results of our proposed studies in which we separated early versus later stages of metastasis. Polyoma cells were introduced via the tail vein with the same experimental strategy as above. To determine effects during early stages of metastasis dox treatment was started 1 week prior to cell injection and continued until 2 days post cell injection. Animals were sacrificed at 2 weeks (PY1) or 5 weeks (PY2) post cell injection. Lungs were harvested and lung metastases quantified. The data again show that increased NF-κB activity in the macrophages results in significantly fewer lung metastases (Figure 2). These results suggested that the anti-tumor effect of increased NF-κB activity was apparent very rapidly (within 2 days) after the cells were introduced via the tail vein.

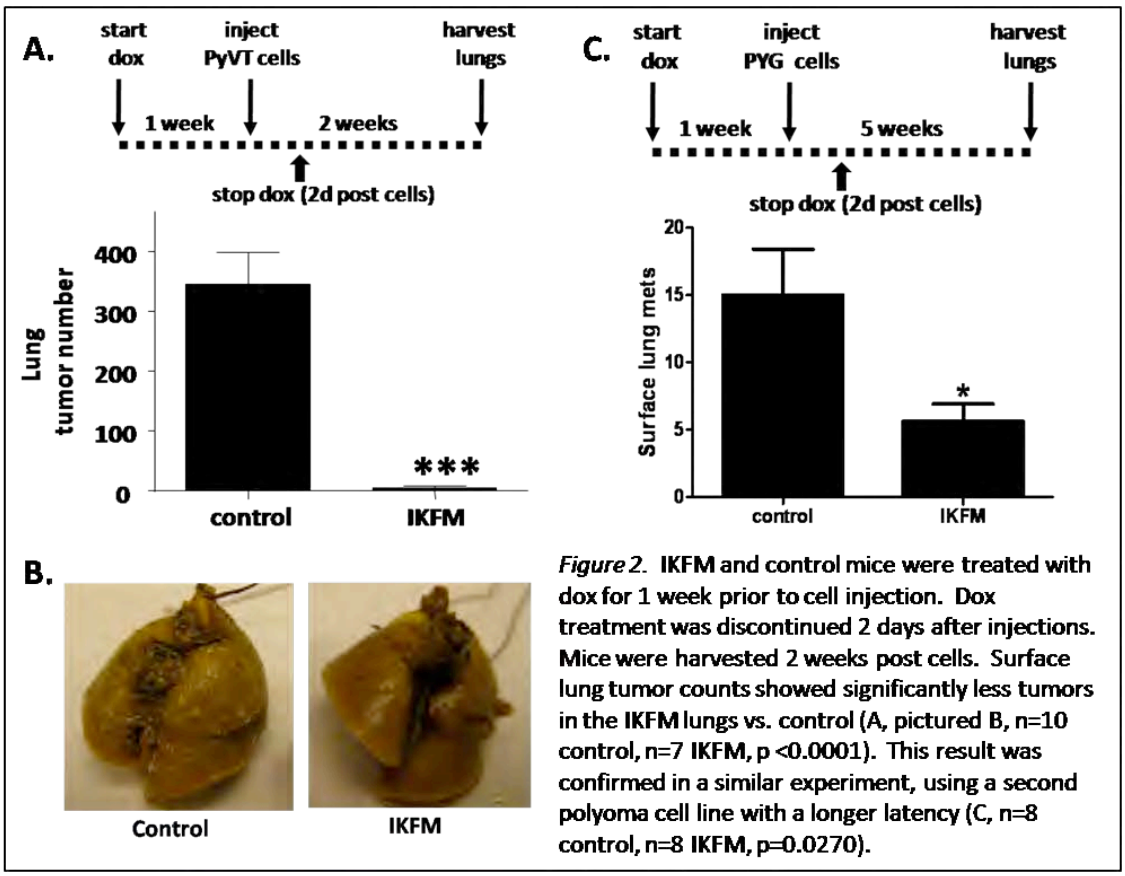


Figure 2. IKFM and control mice were treated with dox for 1 week prior to cell injection. Dox treatment was discontinued 2 days after injections. Mice were harvested 2 weeks post cells. Surface lung tumor counts showed significantly less tumors in the IKFM lungs vs. control (A, pictured B, n=10 control, n=7 IKFM, p <0.0001). This result was confirmed in a similar experiment, using a second polyoma cell line with a longer latency (C, n=8 control, n=8 IKFM, p=0.0270).

*Determine effects of increased NF-κB activity in macrophages at **later stages** of metastasis on lung tumors using a tail vein metastasis model.* The data from the previous two studies demonstrated a strong anti-metastatic effect of increased NF-κB activity. However, our original hypothesis and recently published data from other groups (4-8) is suggestive that NF-κB activity in macrophages can be pro-tumor. The evidence that has been published has been obtained by investigation of tumor-associated macrophages (TAMs). We were thus interested to determine whether we would see this effect if the modulation of NF-κB activity was at a later stage during tumorigenesis when the behavior of the macrophages may be influenced by established metastatic tumors. PY1 or PY3 cells were introduced via the tail vein as above. Dox treatment was started at 2 days post injection. Animals were sacrificed and lungs harvested for quantification of lung metastases at 2 weeks. In these studies no significant impact on tumor numbers was observed (Figure 3).

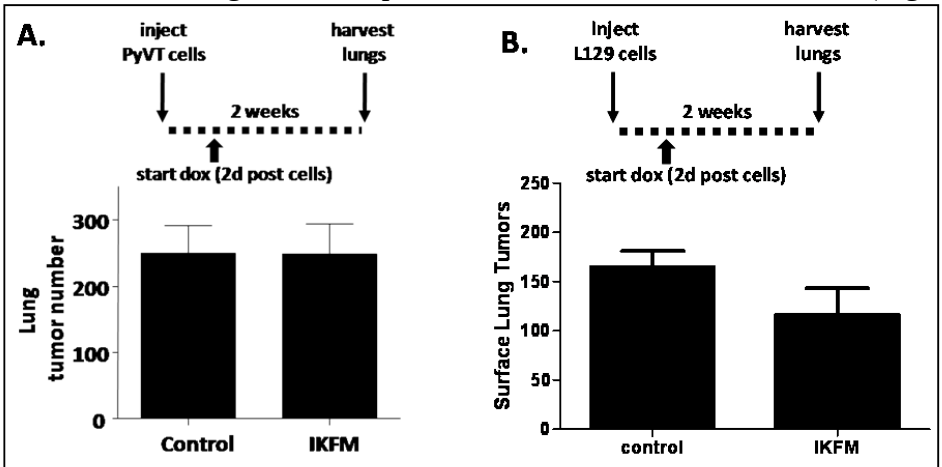
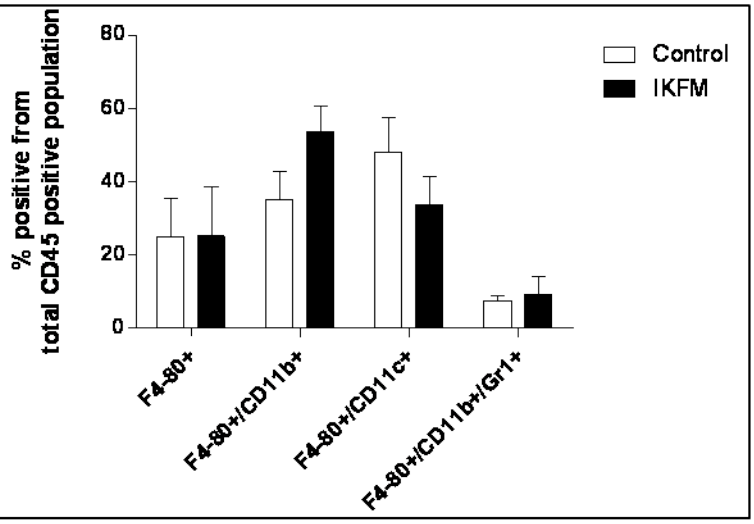


Figure 3. IKFM mice and controls were injected with tumor cells at day 0. Two days post injection, dox treatment began and was continued until the time of sacrifice. Surface lung tumor counts (A) show that there was no significant difference between control and IKFM lung tumor burden (n= 7 control and n=6 IKFM, p =0.9859). This result was confirmed in a separate experiment (B), using a second polyoma cell line (n=10 control, n=11 IKFM, p=0.1540).

This data suggests that at a very early stage in the metastatic process ie. 2 days post introduction of the tumor cells into the tail vein, the anti-metastatic effects of increased NF-κB activity in the macrophages are no longer effective. We were unable to detect any evidence of a pro-tumor effect in these studies. However, the tail vein injection methodology that we are using with introduction of relatively large numbers of aggressive tumor cells that rapidly result in large numbers of lung metastases may be too acute a model to observe pro-tumor effects. Given that different types of macrophages can have different functions we performed Flow analysis of cells harvested from lungs at this end point (Figure 4). At this stage there is no change in the overall numbers of macrophages as defined using the antibody against F4/80. However, within the F4/80 positive population there is a trend towards an increase in the numbers of early stage F4/80/CD11b$^+$ cells versus resident F4/80/CD11c$^+$ populations. As no impact on tumor is observed during the later stages of metastasis we believe that

Figure 4. Flow data from IKFM mice and controls, injected with L129 cells. Dox treatment began two days post injection of cells and continued until time of sacrifice. Flow data reveals an elevated "recruited" macrophage phenotype in the IKFM lungs. (n=3 IKFM, n=3 control, p values not significant).



the changes in macrophage populations are probably greater at earlier stages in the process. It is likely that the macrophages that the tumor cells encounter when they are first injected have the greatest impact in this model.

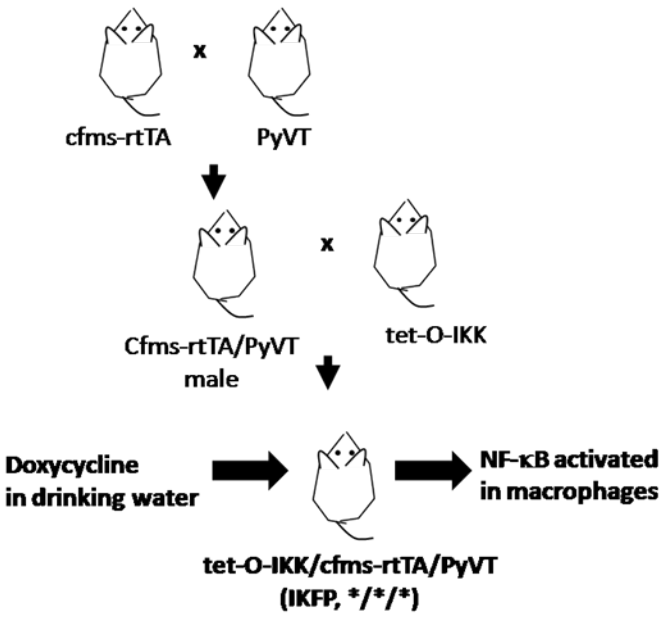


Figure 5. **Breeding strategy for generation of a */*/* IKFP triple transgenic.**

Generate IKFM PyVT mice and determine effects of increased NF-κB activity in macrophages on lung metastasis from a primary mammary tumor. The tail vein metastasis model used above did not display the pro-tumor effect that we were predicting would occur in the presence of TAMs with increased NF-κB activity. To address this question in a model with more established tumors we mated homozygous c-fms rtTA transgenics with PyVT mice to generate double transgenic breeder males. These were mated with IKK females to generate experimental animals including IKFM and PyVT (Figure 5).

PyVT mice rapidly produce multifocal mammary adenocarcinomas together with secondary metastatic tumors in the lung by 12 weeks (9,10). Female triple transgenics and control littermates were palpated twice weekly from 7 weeks until detection of primary palpable mammary tumor. Our original intention was to treat mice with dox (plus littermate controls) from this point until lung harvest 6 weeks later. This strategy was adopted to more closely mimic the full biological process of metastasis from a primary tumor to the lungs. However, treatment of a triple transgenic female with dox resulted in a very detrimental phenotype. The first IKFP triple transgenic after only 28 days of dox treatment appeared lethargic. It had a hunched, feeble appearance, with legs tucked beneath it. The feet and ears were pale yellow in color. The area around the eyes was red and inflamed. Although the mouse had been alone in its cage, the tail had scab marks and was swollen at its base, with unexplained bulges throughout. Upon dissection, mammary tumor burden was average compared to age matched polyoma mice. Gross anatomy of the lungs, kidneys and digestive tract appeared normal. However, the spleen was very enlarged and the liver showed some white, marbled patches. Therefore, at 28 days of treatment we decided to sacrifice the animal for humane reasons and to discontinue this approach. We do not fully understand why in this model induction of increased NF-κB activity in the macrophages makes the animals so sick when we have been able to treat double transgenic mice in the tail vein studies for an equivalent time without observing these effects. We believe that there is some co-operation between the inflammatory state induced by the tumor load in this model and that of the modified macrophages that may establish a pro-inflammatory feed back loop which has these strong pathogenic effects.

Task 2. Determine effects of decreased NF-κB activity in macrophages on lung metastasis:

Determine effects of decreased NF-κB activity in macrophages on lung tumor development using a tail vein metastasis model.

To determine whether the opposite effect was observed when NF-κB is inhibited in the macrophages in our tail vein injection model, PY1 cells were introduced via the tail vein into immunocompetent DNFM and control mice. Experimental subsets included DNFM and control littermates with dox provided at 2mg/ml in 5% sucrose drinking water *ad lib* for this and all following dox induction experiments. Dox treatment started 1 week prior to PY1 cell injection and continued until harvest at

2 weeks post cell injection. At the end point the number of surface lung metastases was quantified (Figure 6A). We detected no significant difference in the numbers of lung metastases in mice in which NF-κB activity was inhibited in the macrophages throughout the study. Given the data obtained from the IKFM mice that suggested that an anti-tumor effect was observed early in the metastatic process and that no effect was seen for modulation of NF-κB from 2 days after introduction of the cells together with our suspicion that this model was not optimal for investigation of later stage TAM-related effects, we decided to concentrate on the time point that was producing the most interesting data ie. dox treatment for 1 week prior to cell introduction until 2 days post cell introduction.

*Determine effects of decreased NF-κB activity in macrophages at **early stages** of metastasis on lung tumors using a tail vein metastasis model.*

The data obtained from studies using the IKFM mice suggests a strong anti-tumor influence of increased NF-κB activity in macrophages at very early stages of the metastatic process. We performed similar studies using the DNFM mice to determine whether these resulted in the opposite effect. PY1, 2 or 3 cells were introduced via the tail vein with the same experimental strategy as above. Dox treatment started 1 week prior to cell injection and continued until 2 days post cell injection. Lungs were harvested at 2 weeks (PY1 and PY3) or 5 weeks (PY2) post cell injection (Figure 6B). The subset of mice that were injected with PY1 demonstrated a significant pro-tumor impact of inhibition of NF-κB activity in the early stage of metastasis. PY3 cells exhibited the same trend but the numbers in the subset of mice that we used in this study did not reach significance (p= 0.1564). We believe that these results represent an indication that inhibition of NF-κB in macrophages during early stages of metastasis can have a pro-tumor impact.

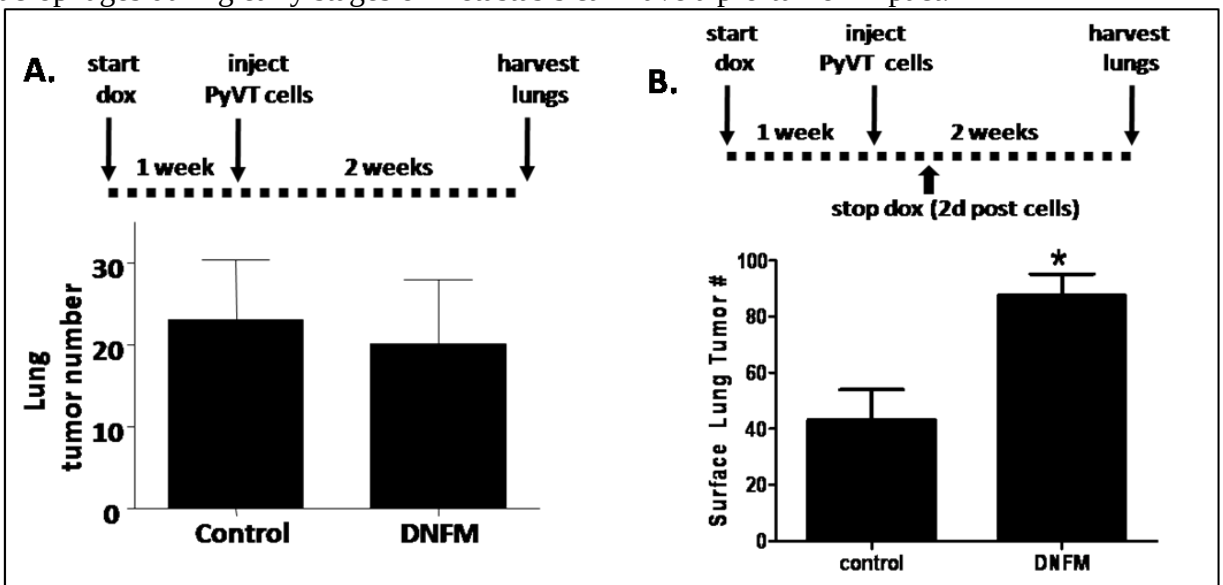


Figure 6. **DNFM mice and controls were treated with dox 1 week prior to cell injection and treatment was continued until the time of sacrifice. Surface lung tumor counts (A) revealed no significant difference in control vs. DNFM (n= 6 controls, n=8 DNFM, p=0.7977). In a separate experiment, DNFM mice and controls were treated with dox 1 week prior to cell injection. Dox treatment was discontinued 2 days after cell injection, and mice were harvested at 2 weeks post cells. Surface lung tumor counts (B) show tumor burden significantly increased in DNFM vs. control (n= 6 control, n=9 DNFM, p = 0.0039).**

*Determine effects of decreased NF-κB activity in macrophages at **later stages** of metastasis on lung tumors using a tail vein metastasis model.*

We originally proposed to investigate the effects on decreasing NF-κB activity in macrophages during the later stages of metastasis using the tail vein model. However, the data that we obtained from our other studies strongly suggests that we would not observe an impact, potentially due to limitations of the model. Therefore, we decided to focus on the other time points.

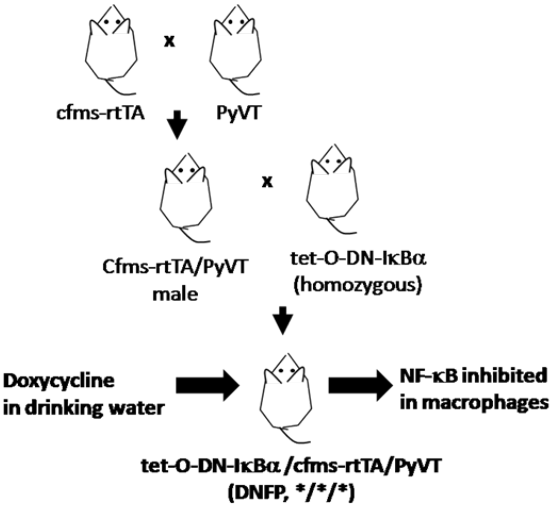


Figure 7. **Breeding strategy for generation of a */*/* DNFP triple transgenic. (*/-/* mice used as controls do not contain the cfms rtTA).**

Generate DNFM PyVT mice and determine effects of increased NF-κB activity in macrophages on lung metastasis from a primary mammary tumor. Our original hypothesis suggested that inhibition of NF-κB activity in macrophages would inhibit mammary to lung metastasis. While the data from our tail vein studies did not support this idea, we were concerned that the tail vein model did not allow investigation of effects in the presence of established tumor. Therefore we mated homozygous c-fms rtTA transgenics with PyVT mice to generate double transgenic breeder males. These were mated with homozygous DN females to generate experimental animals including DNFM and PyVT (Figure 7). Female triple transgenics and littermate controls were palpated twice weekly from 7 weeks until detection of primary palpable mammary tumor. Mice were treated with dox from this point until mammary and lung harvest 30 days later (Figure 8). To attempt to minimize variability in our studies we use littermates as experimental animals and controls. Generation of the triple transgenic mice can be variable within age-matched litters. The first time that we ran through this experimental protocol we believed that we had included 7 triple transgenics and 5 control littermates. However, upon confirming the genotyping at the end of the study we discovered that one mouse had been mis-genotyped and we actually had 8 triple transgenics and 4 control littermates. The data from this set was significant and suggested that inhibition of NF-κB activity in the macrophages in this model resulted in decreased primary mammary tumor load and decreased numbers of lung metastases. This was very intriguing as it suggested that inhibition of NF-κB could be pro-tumor in our tail vein metastasis model but anti-tumor in the model in which a primary tumor is developing. The observed effect on primary tumor would agree with our original hypothesis and with the recently published data from other groups that suggests that in some models NF-κB activity in macrophages has pro-tumor effects. We completed TUNEL staining on mammary sections harvested at the endpoint to quantify levels of apoptosis both over the tissue as a whole and by defining hyperplasia, adenoma and carcinoma as different stages of mammary tumor progression. We did not detect any significant changes in apoptosis between triple transgenics and controls in the tissue as a whole or in defined stages (data not shown).

We were concerned about the relatively low number of mice in the control group so we decided to generate additional mice to increase the numbers in each group (Second Set – Figure 8). The results from this set were not significant. Combining the data from all animals resulted in non-significant results for the group as a whole. We rechecked all the mice from both groups for expression of the DN transgene by RT-PCR and all triple transgenics are expressing the transgene. We must conclude from the available data that there is no significant effect of inhibition of NF-κB as tested in this model. Having three transgenic component parts within the model each with some inherent variability may be obscuring important findings, therefore, we would like to adopt a different strategy to address this question (see conclusions section).

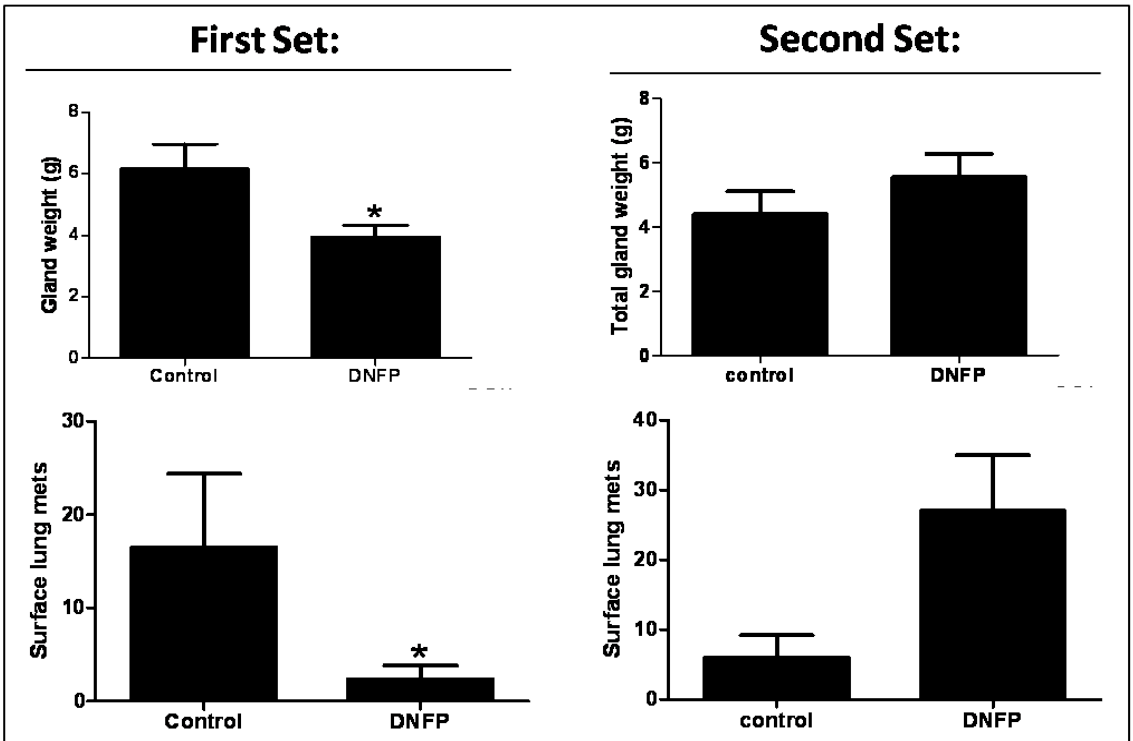


Figure 8. **DNFP triple transgenics and litttermate controls (did not contain cfms-rtta transgene) were palpated until the first primary tumor was found. At that time, mice were put on dox treatment for 30 days, then harvested and mammary glands weighed for a total gland weight. Lungs were inflated with Bouin's fixative and surface lung tumors were counted under a dissecting microscope. Two sets were collected. The first set (n=4 control, n=8 DNFP) showed significant decrease in tumor burden in the DNFP mice vs control (p = 0.0177) as well as significantly reduced lung tumor numbers in DNFP (p=0.0325). However, the second set (n=3 control, n=4 DNFP) did not show a significant difference in either end-point (p=0.311 and p=0.0868 respectively).**

KEY RESEARCH ACCOMPLISHMENTS

During this reporting period;

1) We have investigated the effects of increased NF-κB activity in macrophages in a tail vein metastasis model and obtained unexpected data that suggest that this results in a strong anti-tumor effect but only during early stages of metastasis.
2) Our data using a tail vein metastasis model suggests that decreased NF-κB activity in macrophages during early stages of metastasis can have pro-tumor effects.
3) Our studies combining our inducible macrophage model with the polyoma model of mammary tumorigenesis are inconclusive due in part to technical issues. Our data suggests that prolonged induction of NF-κB in macrophages concurrently with a relatively large primary tumor load may generate a hyper-inflammatory feedback loop. While one group of mice suggested an impact of inhibition of NF-κB on primary tumor development, a second set of mice failed to confirm this observation.

REPORTABLE OUTCOMES

Presentations

Research Assistant 1 Whitney Barham gave invited presentation at the annual Host-Tumor Interactions Program and Department of Cancer Biology 9th annual joint retreat titled "Modulation of NF-κB in Macrophages can produce both pro- and anti-tumor effects during mammary tumor progression"

Abstracts
Poster presentation at the Cancer and Inflammation Keystone Symposium Feb 2010
Poster presentation at the AACR 101st Annual Meeting April 2010
Poster presentation at the Vanderbilt Ingram Cancer Center retreat May 2010
Animal models
We have established the feasibility of using our novel inducible macrophage targeted transgenics to investigate the effects of modulating NF-κB activity in macrophages on mammary tumorigenesis.
Funding applications
BC101825 DOD Breast Cancer Program IDEA application titled "Timing of NF-kappaB modulation during tumor progression is critical for therapeutic outcome". Holder of this grant is PI on submitted proposal.
BC102696 DOD Breast Cancer Program IDEA: Collaborative Option application titled "Development and Testing of Therapeutic Potential of Nanobiotechnology-Targeted siRNA Designed to Inhibit NF-κB Classical & Alternative Signaling in Macrophages". Holder of this grant is Partnering PI on submitted proposal.

CONCLUSIONS
When we initated these studies our hypothesis was that NF-κB activity in macrophages determines metastatic potential and thus represents a target for inhibition of metastatic breast cancer. We were expecting that increasing NF-κB in macrophages would skew them towards a pro-tumor tumor-associated macrophage (TAM) phenotype. We were also predicting that these macrophages would induce a chronic inflammatory environment that would support tumor metastasis by increasing angiogenic and matrix remodeling signals. We proposed to address two questions. 1) Does activation of NF-κB in macrophages contribute to mammary metastasis? 2) Can inhibition of NF-κB in macrophages inhibit mammary to lung metastasis? Our data at this point suggests that the role of NF-κB in macrophages is more complex that we believed. Contrary to our expectations, activation of NF-κB in macrophages at early stages of metastasis appears to exhibit powerful anti-tumor effects. Futhermore, indications are that inhibition of NF-κB in macrophages at this early stage can have pro-tumor impact, and there remains in place evidence that NF-κB in macrophages at later stages may also have pro-tumor effects. It appears that NF-κB activity in macrophages does determine metastatic potential and may represent a target for inhibition of metastatic breast cancer but that the timing of such an intervention during the progression of the tumor may be critical to therapeutic outcome. There are efforts being made to develop inhibitors of NF-κB as potential therapeutics for the treatment of breast cancer. Our data may suggest that inhibition of NF-κB in a patient with circulating metastatic tumor cells may be counter-productive. As our results have taken us in an unexpected direction we have requested a no cost extension of this funding to amend the original SOW as described below to complete studies that are necessary in order to publish these important findings.

Proposed additional studies
Our data suggests that in the tail vein metastasis model macrophages in which NF-κB activity is modulated impact tumor progression rapidly during a short window (potentially within 2 days after introduction of tumor cells). A likely explanation for this observation is that tumor cells are being rapidly deleted by an enhanced innate immune response. We therefore propose to treat IKFM or DNFM double transgenics or control mice with dox for 1 week and harvest prior to cell injection to investigate the macrophage populations and induced microenvironment that the tumor cells encounter upon introduction via the tail vein. We will also treat mice with dox for 1 week, inject tumor cells and sacrifice mice at 1 hour, 6 hours and 2 days post cell injection to determine effects at early stages following tumor challenge. Analysis will include preparation of RNA and Real Time PCR for polyoma expression to investigate the efficiency of seeding to the lungs. This data should provide an indication of whether tumor cells are ablated prior to reaching the lungs or after trapping

within the lungs. Flow analysis will determine changes in inflammatory cell populations together with Real-Time PCR for markers of M1 versus M2 macrophage types to determine if there is a skewing towards anti-tumor M1 macrophages. The level of cell death will be assessed by measuring Caspase-3 levels. As a mechanism leading to rapid cell killing may be the production of ROS, we propose to measure levels of ROS *in vivo* using treatment with luminol.

The data that we have obtained from our triple transgenic studies is inconclusive at this stage. Prolonged treatment of IKFP triple transgenics appears to have major detrimental effects. We propose to adapt this study such that triple transgenics and controls are palpated as previously described until a primary tumor is detected. Then tumors are allowed to progress for a further 20 days followed by dox treatment for a relatively short 10 day period that represents modulation of NF-κB late in the metastatic process. As our data using our DNFP triple transgenics was intriguing but not conclusive we propose a study in which PY1 or PY3 cells are introduced into DNFM double transgenics and control mice to generate a single orthotopic tumor. Following detection of the orthotopic tumor by palpation, mice will be placed on dox for up to 20 days and tumor growth and final tumor burden measured. This strategy will remove the variability inherent in the polyoma transgenic model and provide evidence as to whether inhibition of NF-κB in macrophages impacts primary tumor growth as was suggested by Set 1 of our triple transgenic study (Figure 8). This cumulative data should provide the basis for a strong publication.

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APPENDICES

Abstract for Inflammation and Cancer Keystone Symposium

Activation of NF-κB in macrophages inhibits mammary metastasis to lung in a tail vein model

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Macrophages can exhibit both pro and anti-tumor functions and NF-κB signaling can regulate genes that mediate these effects. However, the role of NF-κB signaling in directly influencing macrophage contribution to mammary tumorigenesis is largely unknown. We have generated a doxycycline inducible transgenic model that enables modulation of NF-κB signaling within macrophages through expression of a constitutively active form of IKK2, the upstream kinase in the classical NF-κB cascade. In this model, the c-fms promoter drives macrophage specific expression of the reverse tetracycline transactivator (rtTA). Administration of doxycycline in drinking water allows the rtTA protein to bind to the Tet operon, driving IKK2 transgene expression and constitutive NF-κB activity within macrophages.

We are using this model in tail vein metastasis studies with mammary tumor cell lines derived from the Polyoma model of mammary tumorigenesis. Our data suggest that increased NF-κB activity within macrophages can enhance host defense and have anti-tumor effects at least during early stages of tumorigenesis. These effects may be mediated by modulation of expression of genes that define M1 versus M2 macrophage types. Although recent data suggests that NF-κB activity within tumor associated macrophages can be pro-tumor, our model argues that at earlier stages of tumor development NF-κB activity within macrophages may have opposite effects.

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Macrophage specific regulation reveals both pro- and anti-tumor effects of NF-kappaB during mammary tumor progression

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An increased level of macrophages in human tumors is associated with a poorer prognosis. The transcription factor Nuclear Factor-kappaB (NF-kappaB) is an important regulator of gene expression within macrophages. Recent studies point to a pro-tumorigenic role for NF-kappaB activity in macrophages; however NF-kappaB also regulates expression of genes such as inducible nitric oxide synthase which are associated with anti-tumor effects. We believe that NF-kappaB activity in macrophages can have both anti- and pro-tumor effects depending on tumor stage.

We have developed novel murine models to determine the effects of modulation of NF-kappaB activity specifically within macrophages during primary mammary tumor growth and metastasis. We have used the cfms promoter to specifically express the reverse tetracycline transactivator (rtTA) protein in macrophages. The rtTA can drive tet operon regulated gene expression in the presence of doxycyline (dox). We have used this with a dominant negative IkappaB alpha (DN-IkappaBalpha) to inhibit NF-kappaB signaling in the Polyoma mouse mammary tumor model, this model is termed DNFP. We have also used the cfms-rtTA in combination with a constitutively active IKK2 transgene to activate NF-kappaB signaling, this model is termed IKFM.

When NF-kappaB is inhibited in macrophages during primary tumor growth in our DNFP model there is a reduction in primary tumor growth accompanied by a reduced level of lung metastasis. This data suggests that NF-kappaB in macrophages is acting in a pro-tumor manner, in agreement with recently published data. In contrast, we have found that activation of NF-kappaB in macrophages by treating IKFM mice with doxycycline for 1 week before and during two weeks following tail vein injection of polyoma mammary tumor cells leads to a significant reduction in surface lung tumor formation. In additional studies, IKFM mice were pretreated with dox for 1 week but treatment was stopped two days after tumor injection. A similar reduction in tumor number was observed, suggesting an inhibition of cell seeding. Real time PCR analysis of lungs from mice with activation of NF-kappaB in macrophages suggests an anti-tumor “M1” macrophage phenotype.

In summary, our data indicates that NF-kappaB activity in macrophages can have both anti- and pro-tumor effects dependent on tumor stage. This data would suggest that cell specificity and timing of treatment are important considerations with regards to the use of NF-kappaB inhibitors in cancer treatment.